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The Mediation of Cyclic AMP in Octopaminergic Modulation at Neuromuscular Junctions of the Mealworm, *Tenebrio molitor*

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ABSTRACT—The possibility that there is mediation of cyclic AMP in the octopaminergic modulation of neuromuscular transmission was examined. Octopamine (1 μM) potentiated the amplitude of neurally-evoked excitatory junctional potentials (EJPs) at the neuromuscular junctions on the ventral longitudinal muscle of the mealworm, *Tenebrio molitor*. Although lower doses of octopamine (0.01 μM) or cyclic AMP (100 μM) had no effects on EJPs, the amplitude of EJPs was potentiated by these compounds when the phosphodiesterase activity was inhibited with simultaneous application of phosphodiesterase inhibitor, isobutyl-1-methylxanthine (IBMX, 10 μM). No effects of octopamine, cyclic AMP or IBMX on the time constant of the decay phase of the EJPs and the resting membrane potentials were observed. The results support the presynaptic mediation of cyclic AMP in octopaminergic modulation at neuromuscular junctions.

INTRODUCTION

Octopamine is a multifunctional biogenic amine, believed to be a neurotransmitter, a neurohormone and a neuromodulator in the insect nervous system [1, 2]. Glutaminergic neuromuscular transmission is well modulated by this amine. Presynaptic action of octopamine enhances the neuromuscular transmission in the locust [3], in *Manduca* [4], resulting in the potentiation of the amplitude of excitatory junctional potentials (EJPs) and contributing to the potentiation of twitch tension [3, 5].

There is growing evidence that adenosine 3′, 5′-cyclic monophosphate (cyclic AMP) plays a role as cellular mediator in the nervous system. Octopamine is widely distributed in insect nervous system [1, 6] and specific increase in cyclic AMP induced by octopamine has been reported in various neural tissues [7–13]. These observations suggest that a broad variety of octopaminergic actions is mediated via a second messenger system [14, 15].

It seems, therefore, that there is mediation of cyclic AMP in the modulatory action of octopamine at neuromuscular junctions in insects. The present work was undertaken to examine the mediation of cyclic AMP by using phosphodiesterase inhibitor, isobutyl-1-methylxanthine (IBMX) [16].

MATERIALS AND METHODS

Larvae of the mealworm (*Tenebrio molitor*) were used. The neuromuscular preparation employed was similar to that described by Yamamoto and Washio [17]. The ventral longitudinal muscle fibers of an abdominal segment were exposed by slitting open the larvae along the dorsal midline and removing the alimentary canal and fat bodies. The dissected animal was mounted on a perfusion chamber made of Sylgard Resin (Dow-Corning Co.) and was continuously perfused with *Tenebrio* saline containing 70 mM NaCl, 30 mM KCl, 14.4 mM $MgCl_2$, 0.6 mM $CaCl_2$, 445 mM glucose and 5 mM HEPES. The pH of this saline was adjusted to 7.2 with NaOH. Octopamine HCl and adenosine 3′, 5′-cyclic monophosphate (cyclic AMP)

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were obtained from Sigma Chemical Co. Isobutyl-1-methylxanthine (IBMX) was obtained from Aldrich Chemical Co. These chemicals were dissolved in the saline.

Intracellular recordings from a ventral muscle fiber were made with a glass microelectrode filled with 3 M KCl (5–10 MΩ). To elicit EJPs, the motor nerve innervating the ventral muscle was stimulated at a point close to the segmental ganglion with square pulses of 0.1 msec duration using a pair of fine Ag-hook electrodes. Sixteen EJPs evoked at 2 Hz were averaged to estimate their amplitude, the time constant of single exponential decay phases and resting membrane potentials. These averaging treatments were performed every one minute.

All experiments were carried out at room temperature (24–26°C).

RESULTS

The effects of treatments were examined by three parameters: the amplitude of EJPs, the time constant of their single exponential decay phase and resting membrane potentials. Neurally-evoked EJPs showed no facilitation properties when they were evoked at 2 Hz. Results obtained are summarized in Table 1. Time constant of single exponential decay phase and resting membrane potentials were unchanged with all treatments.

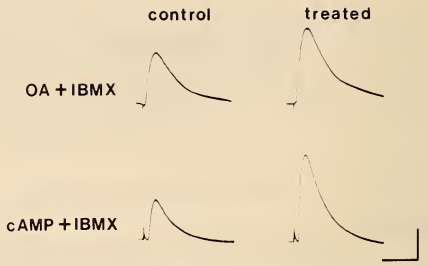

Fig. 1. Action of octopamine, cyclic AMP and IBMX on neurally-evoked EJPs. Sixteen EJPs evoked at 2 Hz were averaged before (control) and 5 min after (treated) the onset of the treatments. Upper two traces were treated with 0.01 μM octopamine and 10 μM IBMX. Lower two traces were treated with 100 μM cyclic AMP and 10 μM IBMX. Calibration: 5 mV, 20 msec.

Phosphodiesterase inhibitor, IBMX, added to perfusate at a concentration of 10 μM (designated as 10 μM IBMX treatment) had no effect on EJP amplitude. Lower doses of octopamine (0.01 μM octopamine treatment) also had no effect. In contrast, these two compounds applied at the same time (0.01 μM octopamine plus 10 μM IBMX treatment) modulated neuromuscular transmission. The amplitude of EJPs was significantly potentiated; the upper two traces in Figure 1 represent this action. The time course comparing 0.01 μM octopamine treatment with 0.01 μM octopamine plus 10 μM IBMX treatment is drawn in Figure 2 A. Treatment by octopamine alone did

Table 1. The effects of octopamine, cyclic AMP and IBMX on the neurally-evoked EJPs

Treatment	EJP amplitude (mV) control/treated	Time constant (ms) control/treated	Resting potential (mV) control/treated	N
OA (1 μM)	8.2±1.7/14.9±2.3*	17.8±4.0/17.5±3.8	−48.6±2.3/−48.1±2.6	5
OA (1 μM) +IBMX (10 μM)	8.2±1.6/13.8±2.3*	16.2±2.4/17.1±2.8	−50.0±1.9/−49.1±2.3	6
OA (0.01 μM)	8.8±1.6/ 8.7±1.3	12.0±0.4/12.8±0.2	−49.2±2.0/−48.7±1.6	5
OA (0.01 μM) +IBMX (10 μM)	7.1±1.4/ 9.9±1.3*	12.0±0.7/13.0±0.6	−48.2±1.5/−48.5±1.9	5
cAMP (100 μM)	12.1±1.1/12.9±0.5	16.0±2.3/15.2±2.2	−49.2±2.0/−48.8±2.2	5
cAMP (100 μM) +IBMX (10 μM)	8.3±1.3/12.3±1.4*	15.5±2.1/14.5±2.3	−49.8±1.4/−48.5±1.3	6
IBMX (10 μM)	8.6±1.2/ 8.8±1.7	13.0±0.4/12.6±0.5	−51.2±2.5/−48.9±2.1	5

Each value is the mean and S.E. just before (control) and 5 min after (treated) the onset of the treatments. * indicates the mean is significantly increased at $P<0.01$ by a paired-sample t-test. OA: octopamine. cAMP: cyclic AMP. IBMX: isobutyl-1-methylxanthine.

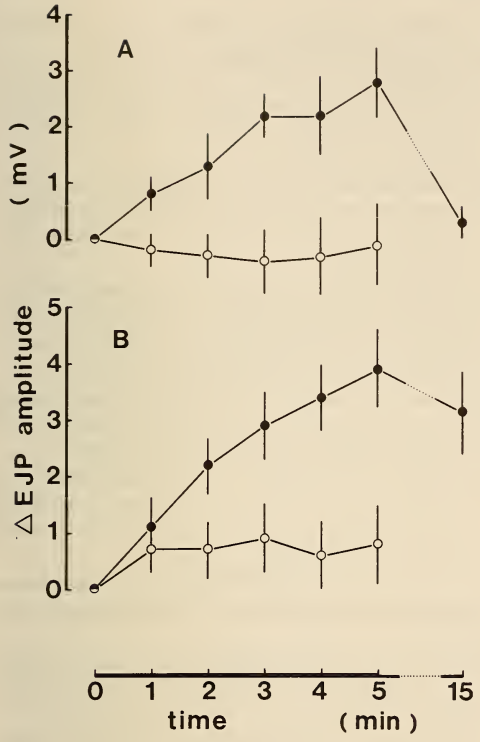

FIG. 2. Time courses of the effect on the EJP amplitude by octopamine, cyclic AMP and IBMX. A: 0.01 μM octopamine (○) and 0.01 μM octopamine plus 10 μM IBMX (●) were added to the perfusate for 5 min. B: 100 μM cyclic AMP (○) and 100 μM cyclic AMP plus 10 μM IBMX (●) were added to the perfusate for 5 min. Each point represents the mean of difference of the EJP amplitude obtained from 5 or 6 determinations; vertical bars represent S.E.

not potentiate EJP amplitude, whereas octopamine plus IBMX resulted in gradual potentiation.

The results were different in the case of octopamine (1 μM) and IBMX(10 μM). EJPs were potentiated both by 1 μM octopamine treatment and 1 μM octopamine plus 10 μM IBMX treatment. Potencies inducing potentiation between these two conditions exhibited the same strength. Amplitude of EJPs increased by 6.7 ± 1.6 mV (n=5) with treatment of 1 μM octopamine alone and by 5.6 ± 0.9 mV (n=6) with 1 μM octopamine plus 10 μM IBMX treatment (means and S.E.) (Table 1). These values were not different by two sample *t*-test.

The action of cyclic AMP had similarities with the potentiating action of octopamine (0.01 μM). One hundred μM cyclic AMP treatment was not effective, whereas 100 μM cyclic AMP plus 10 μM IBMX treatment potentiated the amplitude of EJPs; the lower two traces in Figure 1 represent potentiating action by this treatment. As in the case of 0.01 μM octopamine plus 10 μM IBMX treatment, the time course of potentiating action is drawn in Figure 2B, and also shows the gradual increase by 100 μM cyclic AMP plus 10 μM IBMX treatment.

DISCUSSION

Presynaptic action of octopamine has been revealed by an increase in the frequency of miniature EJPs (MEJPs) at neuromuscular junctions in locust extensor tibiae muscle [3]. Although major changes in cyclic AMP levels occur postsynaptically, octopamine increases cyclic AMP levels in this neuromuscular preparation [12]. Elevation of cyclic AMP levels by phosphodiesterase inhibitors [18, 19] and by a series of cyclic AMP analogues [19] causes an increase in the frequency of MEJPs, showing similarities of action and time course of octopamine on presynaptic terminals. The elevation of cyclic AMP levels by octopamine in the presynaptic terminals is likely to affect transmitter release by the cyclic AMP-dependent protein kinase, changing the calcium levels [20].

At *Tenebrio* neuromuscular junctions, octopamine-induced potentiation of EJP amplitude is contributed to the increase of quantal contents which are estimated by extracellularly-recorded EJP failures, whereas the frequency of MEJPs is not affected by octopamine [21]. These actions suggest the enhancement of calcium accumulation that is associated with the impulse invasion into the presynaptic terminals.

In the present study, the amplitude of EJPs was potentiated by 0.01 μM octopamine plus 10 μM IBMX treatment but not by treatment with 0.01 μM octopamine alone, suggesting that octopamine elevates cyclic AMP levels in the presynaptic terminals. Even 0.01 μM octopamine may elevate cyclic AMP levels; the elevation, however, is so slight that the natural phosphodiesterase inacti-

vates cyclic AMP. If this inactivation is inhibited by IBMX, the elevation is sufficient that cyclic AMP exerts potentiating action. In the case of cyclic AMP treatment, the same event related to phosphodiesterase may occur. The elevation of cyclic AMP by 1 μM octopamine treatment may exceed an optimum level, so that 1 μM octopamine plus 10 μM IBMX treatment potentiates the amplitude of EJPs in the same way as 1 μM octopamine alone.

The mediation of cyclic AMP is also suggested by the similar time courses of octopamine and of cyclic AMP treatments shown in Figure 2, and by similar effects on the amplitude of EJPs but not on the time constant of decay phase or the resting membrane potentials.

Our results support the presynaptic mediation of cyclic AMP in octopaminergic modulation at insect neuromuscular junctions. It is important to point out that neurally-evoked transmitter release is enhanced by the cyclic AMP-mediated octopaminergic action, because this release is closely concerned with muscle contraction.

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